Validation of a Point-of-Care Test Based on RT-LAMP Technology for HCV Detection by Capillary Sampling

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BACKGROUND

Every year, 1.5 million people suffer a new infection by HCV, and nearly 58 million people worldwide live with a Hepatitis C chronic infection. To overcome the underdiagnosis of HCV among hard-to-reach population and accomplishing WHO's target of Hepatitis C virus (HCV) elimination by 2030, it is essential to develop new rapid and easy-to-use molecular diagnostic systems with similar performance values to the gold standard qRT-PCR. Loop-Mediated Isothermal Amplification (LAMP) arises as a promising tool for point-of-care (POC) molecular detection of viral diseases.

In this work, we have validated a new diagnostic tool based on fluorescent reverse transcriptase (RT) LAMP technique, detecting HCV RNA directly from capillary blood from 30 minutes on.

METHODS

SAMPLE PANEL

- 116 HCV+ serum samples
- 13 HCV+ fresh blood samples
- 27 HIV+ controls
- 11 healthy controls

- From 50 µL blood samples, serum was collected by 2 min centrifugation, incubated in lysis buffer for 10 min, and then mixed with RT-LAMP reagents

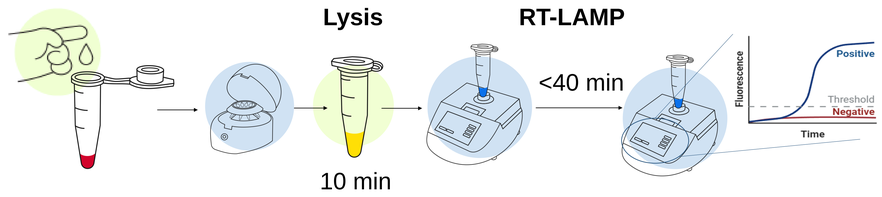
RT-LAMP REACTION

- **Two-well reaction**
  - **HCG124 primers**: GT 1, 2 y 4
  - **HCDN-G3 primers**: GT 3
- **Conditions:**
  - RT → 45ºC
  - LAMP → 68ºC
  - → Sybr-green fluorescence recording

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Point-of-Care RT-LAMP assay detects HCV RNA from capillary blood samples **from 30 minutes**



RESULTS

Serum lysate samples

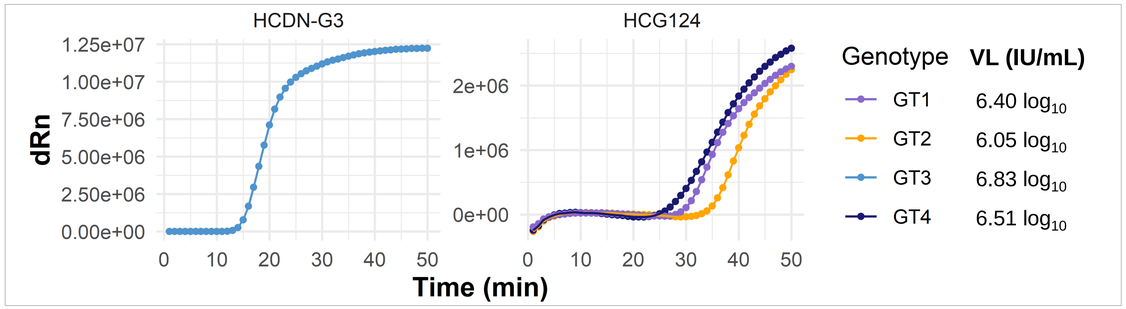


Figure 1. RT-LAMP amplification performance from serum lysates by genotype

Diagnostic sensitivity

Genotype	n/N	
GT1	65/72	90.30%*
GT2	6/6	100%
GT3	20/20	100%
GT4	18/18	100%

* 6/7 undetected simples had a viral load below 3,00 $\log_{10}$ IU/mL

Diagnostic specificity

100%

Limit of detection (LOD)

LOD: 10-20 copies/reaction

Viral load correspondence for blood samples: 3.26 ± 0.20 $\log_{10}$ IU/mL

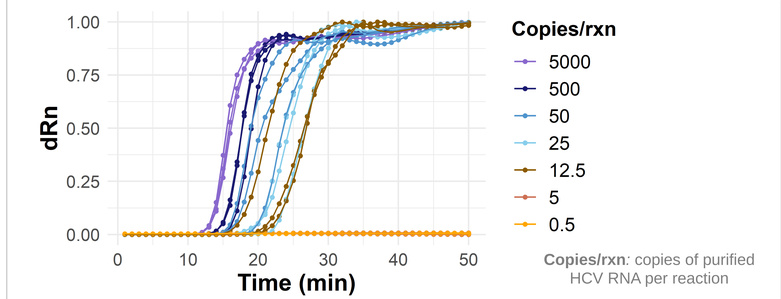


Figure 2. Limit of detection (LOD) of the RT-LAMP assay.

RT-LAMP pre-validation with fresh blood samples:

- Detected: 11/13 HCV positive blood samples

Table 1. Fresh blood sample panel analyzed by RT-LAMP

Sample	Genotype	Viral load (IU/mL)	Amplification time (min)
S01	1a	1,730,000.00	40.10
S02	1b	860,000.00	29.83
S03	1/2/4	9,410,000.00	34.97
S04	1b	198,000.00	38.28
S05	1/2/4	533.00	UD
S06	1/2/4	4,500,000.00	19.76
S07	1a	1,010,000.00	18.99
S09	1/2/4	2,150,000.00	27.12
S10	4	384,000.00	41.8
S11	1a	975,000.00	17.19
S12	1a	243,000.00	22.33
S13	1a	159.00	UD
S14	1a	3,270,000.00	25.14

CONCLUSIONS

- RT-LAMP assay only requires minimal and cheap equipment, with an estimated **price per sample of about 6$**
- These characteristics make RT-LAMP technology a suitable method for isothermal amplification of HCV RNA **from 30 min on.**
- The developed RT-LAMP assay showed **high diagnostic accuracy**, and its analytical sensitivity accomplishes WHO recommendations of an LOD > 3,000 IU/mL for HCV RNA at POC
- The device could be implemented on HCV screening strategies among hard-to-reach populations at POC by capillary punction, taking a step towards **viral hepatitis C elimination**